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I, DAVID DANIEL CLARKE , ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed are true copies of the Provisional specification and drawing(s) as filed on 22 December 1995 in connection with Application No. PN 7274 for a patent by THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH and AMRAD CORPORATION LIMITED filed on 22 December 1995.

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DAVID DANIEL CLARKE
ASSISTANT DIRECTOR PATENT SERVICES

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THE COUNCIL OF THE
QUEENSLAND INSTITUTE OF
MEDICAL RESEARCH and AMRAD
CORPORATION LIMITED

A U S T R A L I A
Patents Act 1990

PROVISIONAL SPECIFICATION
for the invention entitled:

"A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME - II"

The invention is described in the following statement:

A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME - II

The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Vascular endothelial growth factor (hereinafter referred to as "VEGF"), also known as vasoactive permeability factor, is a secreted, covalently linked homodimeric glycoprotein that specifically activates endothelial tissues (Senger *et al.*, 1993). A range of functions have been attributed to VEGF such as its involvement in normal angiogensis including formation of the corpus luteum (Yan *et al.*, 1993) and placental development (Sharkey *et al.*, 1993), regulation of vascular permeability (Senger *et al.*, 1993), inflammatory angiogenesis (Sunderkotter *et al.*, 1994) and autotransplantation (Dissen *et al.*, 1994) and human diseases such as tumour promoting angiogenesis (Folkman & Shing, 1992), rheumatoid arthritis (Koch *et al.*, 1994) and diabetes related retinopathy (Folkman & Shing, 1992).

VEGF is, therefore, an important molecule making it a potentially valuable target for research into therapeutics, prophylactics and diagnostic agents based on VEGF or its activities. There is also a need to identify homologues or otherwise related molecules for use as an alternative to VEGF or in conjunction with VEGF.

In work leading up to the present invention, the inventors sought the multiple endocrine neoplasia type I susceptibility gene (MEN1). Surprisingly, the inventors discovered that a genetic sequence excluded as a candidate for the MEN1 gene was nevertheless a new growth factor having some similarity to VEGF.

Accordingly, one aspect of the present invention comprises a biologically isolated proteinaceous molecule comprising a sequence of amino acids which:

(i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID NO:2; and

(ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

Another aspect of the present invention provides a biologically isolated proteinaceous molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one property in common with VEGF.

A related aspect of the present invention contemplates a biologically isolated proteinaceous molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one of the following properties:

(a) ability to induce proliferation of vascular endothelial cells;

(b) ability to interact with *flt*-1/*flk*-1 family of receptors;

(c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

By "biologically isolated" is meant that the molecule has undergone at least one step of purification from a biological source. Preferably, the molecule is also biologically pure meaning that a composition comprises at least about 20%, more preferably at least about 40%, still more preferably at least about 65%, even still more preferably at least about 80-90% or greater of the molecule as determined by weight, activity or other convenient means, relative to other compounds in the composition. Most preferably, the molecule is sequencably pure.

Another preferred aspect of the present invention provides the molecule in recombinant form.

According to this aspect of the present invention, there is provided a recombinant molecule comprising a sequence of amino acids which:

(i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID NO:2; and

(ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

A related aspect of the present invention is directed to a recombinant molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one property in common with VEGF.

A further related aspect of the present invention contemplates a recombinant molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one of the following properties:

(a) ability to induce proliferation of vascular endothelial cells;

(b) ability to interact with *flt*-1/*flk*-1 family of receptors;

(c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

The present invention also contemplates genomic or partial genome clones encoding a proteinaceous molecule having at least about 15% amino acid similarity but at least about 5% dissimilarity to SEQ ID NO:1.

The amino acid sequence set forth in SEQ ID NO:2 corresponds to human VEGF (referred to herein as "$VEGF_{165}$"). Accordingly, the molecule of the present invention is VEGF-like or is a homologue of VEGF but comprises an amino acid sequence which is similar but non-identical to the amino sequence of VEGF. Although the present invention is exemplified using a human VEGF-like molecule, this is done with the understanding that the instant invention contemplates the homologous molecule and encoding sequence from other mammals such as livestock animals (e.g. sheep, pigs, horses and cows), companion animals (e.g. dogs and cats) and laboratory test animals (e.g. mice, rats, rabbits and guinea pigs) as well as non-mammals such as birds (e.g. poultry birds), fish and reptiles. In a most preferred embodiment, the VEGF-like molecule is of human origin and encoded by a gene located at chromosome 11q13. The present invention extends, therefore, to the genomic sequence or part thereof encoding the subject VEGF-like molecule.

Preferably, the percentage similarity is at least about 30%, more preferably at least about 40%, still more preferably at least about 50%, still even more preferably at least about 60-70%, yet even more preferably at least about 80-95% to all or part of the amino acid sequence set forth in SEQ ID NO:2.

In a particularly preferred embodiment, the VEGF-like molecule of the present invention comprises a sequence of amino acids as set forth in SEQ ID NO:4 or is a part, fragment, derivative or analogue thereof. The amino acid sequence set forth in SEQ ID NO:4 is

also referred to herein as "$SOM175_{short}$". Particularly preferred similarities include about 19-20%, and 29-30%. Reference herein to derivatives also includes splice variants. Accordingly, the present invention extends to splice variants of $SOM175_{short}$. Examples of splice variants contemplated by the present invention include but are not limited to variants with an amino acid sequence substantially as set forth in at least one of SEQ ID NO:6, SEQ ID NO:8 and/or SEQ ID NO:10 or mutants or derivatives or further splice variants thereof.

Another embodiment provides a recombinant molecule having the following characteristics:

(i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one biological property in common with VEGF.

Another embodiment provides a recombinant molecule having the following characteristics:

(i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one biological property in common with VEGF.

Another embodiment provides a recombinant molecule having the following characteristics:

(i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one biological property in common with VEGF.

Another embodiment provides a recombinant molecule having the following characteristics:

(i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one biological property in common with VEGF.

Such properties of VEGF include at least one of:

(a) ability to induce proliferation of vascular endothelial cells;

(b) an ability to interact with *flt*-1/*flk*-1 family of receptors;

(c) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

In accordance with the present invention, a preferred similarity is at least about 40%, more preferably at least about 50% and even more preferably at least about 65% similarity.

Still a further aspect of the present invention contemplates a peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a splice variant thereof such as set forth in SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10 or a chemical equivalent thereof. The biologically isolated or recombinant molecule of the present invention may be naturally glycosylated or may comprise an altered glycosylation pattern depending on the cells from which it is isolated or synthesised. For example, if produced by recombinant means in prokaryotic organisms, the molecule would be non-glycosylated. The molecule may be a full length, naturally occurring form or may be a truncated or otherwise derivatised form.

Yet another aspect of the present invention is directed to a nucleic acid molecule encoding the VEGF-like molecule herein described. More particularly, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or having at least 15% similarity to all or part

thereof or being capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleic acid sequence having at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence as set forth in SEQ ID NO:3. The nucleotide sequence set forth in SEQ ID NO:3 is also referred to herein as "SOM175". Preferably, the percentage dissimilarity is about 35%, more preferably about 39% and even more preferably about 40-50% or greater.

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook *et al* (1989) at pages 9.47-9.51 which is herein incorporated by reference where the washing steps disclosed are considered high stringency. A low stringency is defined herein as being in 4-6X SSC/0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 1-4X SSC/0.25-0.5% w/v SDS at ≥ 45°C for 2-3 hours or high stringent conditions considered herein to be 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

The present invention further contemplates a nucleic acid molecule which encodes a VEGF-like molecule as hereinbefore described having at least 15% nucleotide sequence homology to SEQ ID NO:3. Preferred levels of homology include at least about 40%, more preferably around 60-70%.

The present invention is further directed to the murine homologue of human VEGF (referred to herein as "mVRF"). The mVRF has approximately 85% identity and 92% conservation of amino acid residues over the entire coding region compared to human VEGF. The mVRF is encoded by a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in Figure 9.

The VEGF-like molecule of the present invention will be useful in the development of a range of therapeutic and/or diagnostic applications alone or in combination with other molecules such as VEGF. The present invention extends, therefore, to pharmaceutical

compositions comprising the VEGF-like molecule or parts, fragments, derivatives, homologues or analogues thereof together with one or more pharmaceutically acceptable carriers and/or diluents. Furthermore, the present invention extends to vectors comprising the nucleic acid sequence set forth in SEQ ID NO:3 or having at least about 15%, more preferably about 40% and even more preferably around 60-70% similarity thereto but at least 30% and more preferably around 39% dissimilarity thereto and host cells comprising same. In addition, the present invention extends to ribozymes and antisense molecules based on SEQ ID NO:3 as well as neutralizing antibodies to the VEGF-like molecule. Such molecules may be useful in ameliorating the effects of, for example, over expression of VEGF-like genes leading to angiogenesis or vascularization of tumours.

The present invention also contemplates antibodies to the VEGF-like molecule or nucleic acid probes to a gene encoding the VEGF-like molecule which are useful as diagnostic agents.

The present invention is further described by reference to the following non-limiting Figures and/or Examples.

In the Figures:

Figure 1 Nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence [SEQ ID NO:2] of $VEGF_{165}$.

Figure 2 Nucleotide sequence [SEQ ID NO:3] and corresponding amino acid sequence [SEQ ID NO:4] of SOM175.

Figure 3 Results of BLAST search with SOM175 protein sequence.

Figure 4 BESTFIT alignment of VEGF cDNA and SOM175 cDNA.

Figure 5 Multiple alignment of $VEGF_{165}$ with SOM175 and its splice variants at the nucleotide level.

Figure 6 Multiple alignment of $VEGF_{165}$ with SOM175 and its splice variants at the amino acid level.

Figure 7 Diagrammatic representation of SOM175 and its splice variants.

Figure 8(a) Diagrammatic representation of genomic structure of human SOM175 genomic showing exon/intron map.

Figure 8(b) Diagrammatic representation of genomic structure of human SOM175 showing exon/intron boundries.

Figure 9 Nucleotide and predicted peptide sequences derived from mVRF cDNA clones. Numbering of nucleotides are given on the left, starting from the A of the initiation codon. Amino acids are numbered on the right, starting from the first residue of the predicted mature protein after the putative signal peptide has been removed. The alternately spliced region is double underlined and the resulting peptide sequence from each mRNA is included. A potential polyadenylation signal is indicated in boldface. Start and stop codons of $mVRF_{167}$ and $mVRF_{186}$ are underlined and a polymorphic AC repeat in the 3′ UTR is indicated by a stippled box. The positions of intron/exons boundaries are indicated by arrowheads.

Figure 10 BESTFIT alignments of human and murine VRF protein isoforms. A: $mVRF_{167}$ and $hVRF_{167}$. B: $mVRF_{186}$ and $hVRF_{186}$ from the point where the sequences diverge from the respective 167 amino acid isoforms. Amino acid identities are marked with vertical bars and conserved amino acids with colons. An arrow marks the predicted signal peptide cleavage site of human and mouse VRF.

Figure 11 BESTFIT alignment of $mVRF_{167}$ and $mVEGF_{188}$ (Breier *et al*, 1992) peptide sequences. An arrow marks the signal peptide cleavage site of mVEGF. Identical amino acids are indicated by vertical bars and conservative substitutions by colons. Numbering of amino acids is as described in the legend to Figure 9.

Figure 12 Comparison of gene structure between VRF (a generic VRF gene is shown since the intron/exon organisation of the mouse and human homologues is almost identical) and other members of the human VEGF/PIGF/PDGF gene family. Exons are represented by boxes. Protein coding regions and untranslated regions are shown by filled and open sections respectively. The hatched region in VRF indicates the additional 3′ UTR sequence formed by alternate splicing of the VRF_{186} isoform. Potential alternate splice products of each gene are shown.

Figure 13 Autoradiogram of a Northern blot of total RNA from various adult mouse tissues (as indicated) hybridised with an mVRF cDNA clone. A major transcript of 1.3 kb was detected in all samples.

Figure 14 Film autoradiographs (A-C) and dark-field micrographs (D-E) illustrating the expression pattern of mVRF and mRNA in the mouse. In the E14 mouse embryo (A) positive signals are present over the developing heart (Ha) and cerebral cortex (Cx). A low background signal is also present over other tissues in the section. In the E17 embryo (B) and the heart (Ha) is clearly visible due to a strong hybridisation signal. An equally strong signal is present over brown adipose tissue (Fa) in the back and around the thoracic cage. A moderate hybridisation signal is present over the spinal cord (SC) and the tongue (T). The background signal is reduced compared with the E14 embryo. In the young adult mouse (C-D), positive signals are present over the heart (Ha) and adipose tissue (Fa) around the thoracic cage, while, for example, the lungs (Lu) are unlabeled). The hybridisation signal over the heart is evenly distributed over the entire left ventricle, including papillary muscles (D). In the E17 heart hybridised with an excess of cold probe, no positive signal is present (E). Scale bars = 0.5 mm (A), 1.2 mm (B), 1 mm (C), 0.3 mm (D), 0.1 mm (E).

Figure 15 Dark - (A and C) and bright-field (B and D) micrographs showing mVRF mRNA expression in mouse adipose tissue (A-B) and spinal cord (C-D). A strong hybridisation signal is present over fat (A), as shown by the strong labeling in Sudan black stained sections (B). A weak signal is present also in skeletal muscle (M in A-B). In the adult spinal cord (C) the mVRF probes gave a neuronal staining pattern over the gray matter. Toloudine counterstaining showing that motoneurons in the ventral horn (D), interneurons in the deep part of the dorsal horn and around the central canal (not shown) where largely positive for mVRF mRNA. Scale bars = 0.1 mm (A), 0.1 mm (B), 0.25 mm (C), 0.015 mm (D).

TABLE 1

SUMMARY OF SEQUENCE IDENTITY NUMBERS

SEQ ID NO:1	Nucleotide sequence of $VEGF_{165}$
SEQ ID NO:2	Amino acid sequence of $VEGF_{165}$
SEQ ID NO:3	Nucleotide sequence of SOM175 (VEGF-like molecules)
SEQ ID NO:4	Amino acid sequence of SOM175
SEQ ID NO:5	Nucleotide sequence of SOM175 absent exon 6
SEQ ID NO:6	Amino acid sequence of SOM175 absent exon 6
SEQ ID NO:7	Nucleotide sequence of SOM175 absent exon 6 and exon 7
SEQ ID NO:8	Amino acid sequence of SOM175 absent exon 6 and exon 7
SEQ ID NO:9	Nucleotide sequence of SOM175 absent exon 4
SEQ ID NO:10	Amino acid sequence of SOM175 absent exon 4
SEQ ID NO:11	Oligonucleotide
SEQ ID NO:12	Oligonucleotide
SEQ ID NO:13	Oligonucleotide
SEQ ID NO:14	Oligonucleotide

EXAMPLE 1

Human cDNA clones

The original SOM175 cDNA was isolated by screening a human foetal brain library (λzapII, Stratagene) with the cosmid D11S750 (Larsson *et al,* 1992). The plasmid was excised *"in vivo"* and a single 1.1kb cDNA was obtained. Three independent SOM175 cDNAs clones were also isolated from a human foetal spleen library (Strategane, Uni-zap) using the above-mentioned SOM175 insert as a probe. Three clones were obtained: SOM175-4A, -5A and -6A. SOM175-5A is an alternately spliced clone with exon 4 being absent (SOM175-e4). These library screens were performed using hybridisation conditions recommended by the manufacturer of the library (Stratagene) and random primed insert of SOM175.

Two partial human SOM175 cDNAs have also isolated from a λGT11 human melanoma cell line A2058 library (Clontech) cDNA library screens were performed using hybridisation conditions described by Church and Gilbert, 1984). In each case, the probe was generated by random priming of a PCR product derived from SOM175 (18f-700r).

Mouse cDNA Clones

Human SOM175 was also used to screen a mouse neonatal whole brain cDNA library (Unizap, Stratagene). Four non-chimeric clones were isolated: M175-A, B, C, D. All clones were partial cDNAs and M175-C contained several introns. Three of these cDNAs lacked the exon 6.

Another clone referred to as M1 was completely sequenced and was found to contain the full open reading frame plus part of the 5'utr and total 3'utr.

EXAMPLE 2

DNA SEQUENCE ANALYSIS

The entire sequence of the cDNA clone (SOM175) was compiled and is shown in Figure 2 with its corresponding amino acid sequence. This sequence was screened for open reading frames using the MAP program (GCG, University of Wisconsin). A single open reading frame of 672bp was observed (see Figure 2). There appears to be little 5′ untranslated sequences (2bp). The 3′ untranslated region appears to be complete as it includes a poly-adenylation signal and poly-A tail.

Database homology searches were performed using the BLAST algorithm (run at NCBI, USA). This analysis revealed homology to several mammalian forms of VEGF (see Figure 3). The amount of homology between SOM175 and human $VEGF_{165}$ was determined using the BESTFIT program (GCG, University of Wisconsin; see Figures 4 and 5). Nucleotide homology was estimated at 69.7% and protein homology was estimated as at least 33.3% identity and 52.5% conservation using BESTFIT analysis. BLAST analysis on nucleotide sequences revealed the almost complete match to a human expressed sequence tag EST06302 (Adams *et al.*, 1993).

These data indicate that SOM175 encodes a growth factor that has structural similarities to VEGF. Both genes show start and stop codons in similar positions and share discrete blocks of homology. All 8 cysteines as well as a number of other VEGF residues believed to be involved in dimerisation are conserved. These residues are Cysteine-47, Proline-70, Cysteine-72, Valine-74, Arginine-77, Cysteine-78, Glycine-80, Cysteine-81, Cysteine-82, Cysteine-89, Proline-91, Cysteine-122 and Cysteine-124 and are shown in Figure 6. Given the structural conservation between VEGF and the SOM175 gene product it is also possible that they share functional similarities. It is proposed that SOM175 encodes a VEGF-like molecule that shares some properties with VEGF but has unique properties of its own. The nucleotide sequence and corresponding amino acid sequence of $VEGF_{165}$ is shown in Figure 1.

EXAMPLE 3

The percentage similarity and divergence between $VEGF_{165}$ family and SOM175 family (protein) were analysed using the Clustal method, MegAlign Software, DNASTAR, Wisconsin. The results are shown in Tables 2.1 and 2.2. The alternatively spliced forms of SOM175 are abbreviated to SOM715-e6 where all of exon 6 is deleted; SOM715-e6 and 7 where all of exons 6 and 7 are deleted; and SOM175-e4 where all of exon 4 is deleted. The spliced form of SOM175 are shown in Figure 7. Genomic maps of SOM175 showing intron/exon boundaries are shown in Figure 8a and 8b.

Table 2.1

A Percent nucleotide similarity between splice variants of SOM175 and human $VEGF_{165}$

	$VEGF_{165}$	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
$VEGF_{165}$	***	34.9	39.7	41.4	37.0
SOM175		***	98.9	95.1	99.2
SOM175-e6			***	98.8	84.0
SOM175-e6&7				***	80.3
SOM175-e4					***

B Percent nucleotide divergence between splice variants of SOM175 and human $VEGF_{165}$

	$VEGF_{165}$	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
$VEGF_{165}$	***	41.7	41.6	41.7	41.8
SOM175		***	0.2	0.2	0.0
SOM175-e6			***	0.0	0.2
SOM175-e6&7				***	0.3
SOM175-e4					***

Table 2.2

A Percent amino acid identity between splice variants of SOM175 and human $VEGF_{165}$

	$VEGF_{165}$	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
$VEGF_{165}$	***	31.4	42.3	33.5	40.6
SOM175		***	74.7	73.7	99.1
SOM175-e6			***	76.8	99.1
SOM175-e6&7				***	99.1
SOM175-e4					***

B Percent amino acid divergence between splice variants of SOM175 and human $VEGF_{165}$

	$VEGF_{165}$	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
$VEGF_{165}$	***	65.7	55.4	54.6	57.4
SOM175		***	19.9	4.2	0.0
SOM175-e6			***	0.0	0.0
SOM175-e6&7				***	0.0
SOM175-e4					***

EXAMPLE 4

BIOASSAYS TO DETERMINE THE FUNCTION OF SOM175

Assays are conducted to evaluate whether SOM175 has similar activities to VEGF on endothelial cell function, angiogenesis and wound healing. Other assays are performed based on the results of receptor binding distribution studies.

Assays of endothelial cell function

Endothelial cell proliferation. Endothelial cell growth assays as described in Ferrara & Henzel (1989) and in Gospodarowicz *et al* (1989).

Vascular permeability assay. This assay, which utilises the Miles test in guinea pigs, will be performed as described in Miles & Miles (1952).

Cell adhesion assay. The influence of SOM175 on adhesion of polymorphs to endothelial cells is analysed.

Chemotaxis. This is performed using the standard Boyden chamber chemotaxis assay.

Plasminogen activator assay. Endothelial cells are tested for plasminogen activator and plasminogen activator inhibitor production upon addition of SOM175 (Pepper *et al* (1991)).

Endothelial cell migration assay. The ability of SOM175 to stimulate endothelial cells to migrate and form tubes is assayed as described in Montesano *et al* (1986).

Angiogenesis Assay

SOM175 induction of an angiogenic response in chick chorioallantoic membrane is evaluated as described in Leung *et al* (1989).

Possible neurotrophic actions of SOM175 are assessed using the following assays:

Neurite outgrowth assay and gene induction (PC12 cells)

PC12 cells (a phaeochromocytoma cell line) respond to NGF and other neurotrophic factors by developing the characteristics of sympathetic neurons, including the induction of early and late genes and the extension of neurites. These cells are exposed to SOM175 and their response monitored (Drinkwater *et al* (1991); and Drinkwater *et al* (1993)).

Cultured neurons from the Peripheral Nervous System (PNS)

Primary cultures of the following PNS neurons are exposed to SOM175 and monitored for any response:

- sensory neurons from neural crest and dorsal root ganglia
- sympathetic neurons from sympathetic chain ganglia
- placode derived sensory neurons from nodose ganglia
- motoneurons from spinal cord

The assays are described in Suter *et al* (1992) and in Marinou *et al* (1992).

Where an *in vitro* response is observed, *in vivo* assays for properties such as uptake and retrograde transport are performed as described in Hendry *et al* (1992).

Nerve regeneration (PNS)

Where neurotrophic effects of SOM175 are observed, its possible role in the regeneration of axotomised sensory neurons, sympathetic neurons and motoneurons is analysed by the methods of Otto *et al* (1989); Yip *et al* (1984) and Hendry *et al* (1976).

Actions of SOM175 on CNS neurons

The ability of SOM175 to promote survival of central nervous system neurons is analysed as described in Hagg *et al* (1992); Williams *et al* (1986); Hefti (1986) and Kromer (1987).

Wound Healing

The ability of SOM175 to support wound healing are tested in the most clinically relevant model available, as described in Schilling *et al* (1959) and utilised by Hunt *et al* (1967).

The Haemopoietic System

A variety of *in vitro* and *in vivo* assays on specific cell populations of the haemopoietic system are available and are outlined below:

Stem Cells

Murine

A variety of novel *in vitro* murine stem cell assays have been developed using FACS-purified cells:

(a) Repopulating Stem Cells

These are cells capable of repopulating the bone marrow of lethally irradiated mice, and have the Lin^-, Rh^{hi}, Ly-6A/E^+, c-kit^+ phenotype. The test substance is tested on these cells either alone, or by co-incubation with multiple factors, followed by measurement of cellular proliferation by ^3H thymidine incorporation.

(b) Late Stage Stem Cells

These are cells that have comparatively little bone marrow repopulating ability but can generate D13 CFU-S. These cells have the Lin^-, Rh^{hi}, Ly-6A/E^+, c-kit^+ phenotype. The test substance is incubated with these cells for a period of time, injected into lethally irradiated recipients, and the number of D13 spleen colonies enumerated.

(c) Progenitor-Enriched Cells

These are cells that respond *in vitro* to single growth factors, and have the Lin^-, Rh^{hi}, Ly-6A/E^+, c-kit^+ phenotype. This assay will show if SOM175 can act directly on haemopoietic progenitor cells. The test substance is incubated with these cells in agar cultures, and the number of colonies enumerated after 7-14 days.

Atherosclerosis

Smooth muscle cells play a crucial role in the development or initiation of atherosclerosis, requiring a change in their phenotype from a contractile to a synthetic state. Macrophages, endothelial cells, T lymphocytes and platelets all play a role in the development of atherosclerotic plaques by influencing the growth and phenotypic modulations of smooth muscle cell. An *in vitro* assay that measures the proliferative rate and phenotypic modulations of smooth muscle cells in a multicellular environment is used to assess the effect of SOM175 on smooth muscle cells. The system uses a modified Rose chamber in which different cell types are seeded onto opposite coverslips.

Effects of SOM175 on bone

The ability of SOM175 to regulate proliferation of osteoblasts is assayed as described in Lowe *et al* (1991). Any effects on bone resorption are assayed as described in Lowe *et al* (1991). Effects on osteoblast migration and changes in intracellular molecules (e.g. cAMP accumulation, alkaline phosphatase levels) are analysed as described in Midy *et al* (1994).

Effects on skeletal muscle cells

Effects of SOM175 on proliferation of myoblasts and development of myotubes can be determined as described by Ewton *et al* (1980) and by Gospodarowicz *et al* (1976).

EXAMPLE 5

CLONING MURINE VEGF DNA

Isolation of cDNAs

Murine VRF (mVRF) clones were selected from a lambda Zap new born whole brain cDNA library (Stratagene). Primary phage from high density filters (5 x 10^4 pfu/plate) were identified by hybridisation with a 682bp ^{32}P-labelled probe generated by PCR from an hVRF cDNA (pSOM175) as described above. Hybridisation and stringent washes of nylon membranes (Hybond-N) were carried out at 65°C under conditions described by Church and Gilbert (1984). Positive plaques were picked, purified and excised *in vivo* to produce bacterial colonies containing cDNA clones in pBluescript SK-.

Isolation of genomic clones

Genomic clones were isolated from a mouse strain SV/129 library cloned in the lambda Fix II vector (Stratagene). High density filters (5 x 10^4 pfu/filter) were screened with a 563 bp ^{32}P-labelled probe generated by PCR amplification of the nucleotide 233-798 region of the mVRF cDNA (see Figure 9). Positive clones were plugged and re-screened with filters containing 400-800 pfu. Large scale phage preparations were prepared using the QIAGEN lambda kit or by $ZnCl_2$ purification (Santos, 1991).

Nucleotide sequencing and analysis

cDNAs were sequenced on both strands using a variety of vector-based and internal primers with Applied Biosystems Incorporated (ABI) dye terminator sequencing kits according to the manufacturer's specifications. Sequences were analysed on an ABI Model 373A automated DNA sequencer. Peptide homology alignments were performed using the program BESTFIT (GCG, Wisconsin).

Identification of intron/exon boundaries

Identification of exon boundaries and flanking regions was carried out using PCR with mouse genomic DNA or mVRF genomic lambda clones as templates. The primers used in PCR to identify introns were derived from the hVRF sequence and to allow for potential human-mouse sequence mismatches annealing temperatures 5-10°C below the estimated T_m were used. All PCR products were sized by agarose gel electrophoresis and gel purified using QIAquick spin columns (Qiagen) and the intron/exon boundaries were sequenced directly from these products. In addition, some splice junctions were sequenced from subcloned genomic fragments of MVRF. Intron/exon boundaries were identified by comparing cDNA and genomic DNA sequences.

Northern analysis

Total cellular RNA was prepared from a panel of fresh normal adult mouse tisues (brain, kidney, liver, muscle) using the method of Chomczynski and Sacchi (1987). 20μg of total RNA were electrophoresed, transferred to a nylon membrane (Hybond N, Amersham) and hybridised under standard conditions (Church & Gilbert, 1984). Filters were washed at 65°C in 0.1xSSC (20xSSC is 3M NaCl/0.3M trisodium citrate), 0.1% SDS and exposed to X-ray film with intensifying screens at -70°C for 1-3 days.

Characterisation of mVRF cDNAs

Murine VRF homologues were isolated by screening a murine cDNA library with an hVRF cDNA clone. Five clones of sizes varying from 0.8-1.5 kb were recovered and sequenced. The cDNA sequences were complied to give a full length 1041 bp cDNA sequence covering the entire open reading frame (621 bp or 564 bp depending on the splice form, see below) and 3' UTR (379 bp), as well as 163 bp of the 5' UTR (Figure 9).

The predicted initiation codon matched the position of the start codon in hVRF. One other out of frame ATG was located at position -47 and two termination codons were observed upstream (positions -9 and -33, respectively) and in-frame with the putative

initiation codon.

The predicted N-terminal signal peptide of hVRF appears to be present in mVRF with 81% identity (17/21 amino acids). Peptide cleavage within mVRF is expected to occur after reside 21 (Figure 10). These data suggest that mature mVRF is secreted and could therefore conceivably function as a growth factor.

As with hVRF, two open reading frames (ORFs) were detected in cDNAs isolated by library screening. Four of five clones were found to be alternatively spliced and lacked a 101 bp fragment homologous to exon 6 of hVRF. The predicted peptide sequences of the two isoforms of mVRF were determined and aligned with the corresponding human isoforms (Figure 10).

The message encoding $mVRF_{186}$ contains a 621 bp ORF with coding sequences terminating at position +622, towards the end of exon 7 (Figure 9). The smaller message encoding $mVRF_{167}$ actually terminates downstream of the +622 TAG site due to a frame shift resulting from splicing out of the 101 bp exon 6 and the introduction of a stop codon (TGA) at position +666, near the beginning of exon 8 (Figure 9).

The $mVRF_{186}$ protein has strong homology to the amino and central portions of VEGF while the carboxyl end is completely divergent an is alanine rich. $mVRF_{167}$ possesses these similarities and also maintains homology to mVEGF right through to the C-terminus (Figure 11). The overall homology of $mVRF_{167}$ to $hVRF_{167}$ was 85% identity and 92% similarity, respectively (Figure 10). Likewise, homology between $mVRF_{167}$ and mVEGF (Breier *et al*, 1992) was 49% identity and 71% conservative amino acid substitution, respectively (Figure 11).

A canonical vertebrate polyadenylation signal (AATAAA) (Birnstiel *et al*, 1986) was not present in the mVRF cDNA, however, the closely matching sequence GATAAA is present at similar positions in both mouse and human VRF cDNAs (Figure 9). In contrast to hVRF, mVRF was found to contain an AC dinucleotide repeat at the

extreme 3′ end of the 3′ UTR (nucleotide positions 998 to 1011, Figure 9). Polymorphism of this repeat region was observed between some of the mVRF cDNAs, with the number of dinucleotides varying from 7 to 11.

Genomic characterisation of mVRF

Intron/exon boundaries (Table 3) were mapped using primers which flanked sequences homologous to the corresponding hVRF boundaries. Introns I, III, IV and VI of mVRF (Table 3, Figure 12) were smaller than the hVRF intervening sequences. The complete genomic sequence was compiled from the 5′ UTR of mVRF through to intron VI, the largest intervening region (2.2 kb), by sequencing amplified introns and cloned genomic portions of mVRF. There was only one major difference in genomic structure between mVRF and hVRF and that was the exon 7/intron VI boundary of mVRF was located 10bp further downstream in relation to the cDNA sequence, hence exon 7 in mVRF is 10bp longer than the corresponding exon in hVRF.

Exons 6 and 7 are contiguous in mVRF, as has been found to occur in the human homologue. The strong sequence homology between exon 6 of mVRF and hVRF (Figure 10) suggests that this sequence is not a retained intronic sequence but rather encodes a functional part of the VRF_{186} isoform.

General intron/exon structure is conserved between the various members of the VEGF gene family (VEGF, PIGF, hVRF) and therefore it is not surprising that the overall genomic organisation of the mVRF gene is very similar to these genes (Figure 12).

Previous comparative mapping studies have shown that the region surrounding the human multiple endocrine neoplasia type 1 disease locus on chromosome 11q13 is syntenic with the proximal segment of mouse chromosome 19 (Rochelle *et al*, 1992). Since the inventors have mapped the hVRF gene to within 1kb of the human *MEN1* locus (see above) it is most likely that the murine VRF gene maps near the centromere of chromosome 19.

Expression studies of mVRF

Northern analysis of RNA from adult mouse tissues (muscle, heart, lung and liver) showed that expression appears to be ubiquitous and occurs primarily as a major band of approximately 1.3kb in size (Figure 14). This is somewhat different to the pattern observed for hVRF in which two major bands of 2.0 and 5.5 kb have been identified in all tissues examined. The 1.3 kb murine message presumably corresponds to the shorter of the human transcripts and the size variation thereof is most likely due to a difference in the length of the respective 5′ UTRs.

EXAMPLE 6

EXPRESSION OF MURINE VEGF IN PRE- AND POST-NATAL MOUSE

Animals

Timed pregnant (n=4) and young adult (n=2) mice (C57 inbred strain, ALAB, Sweden) were sacrificed with carbon dioxide, and the relevant tissues were taken out and frozen on a chuck. Tissues were kept at -70°C until further use. Two gestational ages was used in this study; embryonic day 8 (E8), 14 and E17.

***In situ* hybridisation histochemistry**

In situ hybridisation was performed as previously described (Dagerlind *et al*, 1992). Briefly, transverse sections (14μm) were cut in a cryostat (Microm, Germany), thawed onto Probe-On slides (Fisher Scientific, USA) and stored in black sealed boxes at -70°C until used. The sequences of the synthetic 42-mer oligonucleotides complementary to mRNA encoding mVRF were ACCACCACCTCCCTGGGCTGGCATGTGGCACGTGCATAAACG [SEQ ID NO:11] (complementary to nt 120-161) and AGTTGTTTGACCACATTGCCCATGAGTTCCATGCTCAGAGGC [SEQ ID NO:12] (complementary to nt 162-203). To detect the two alternative splice forms oligonucleotide GATCCTGGGGCTGGAGTGGGATGGATGATGTCAGCTGG [SEQ ID NO:13] (complementary to nt xxx-xxx) and GCGGGCAGAGGATCCTGGGGCTGTCTGGCCTCACAGCACT [SEQ ID NO:14] were used. The probes were labeled at the 3′-end with deoxyadenosine-alpha[thio]triphosphate [^{35}S] (NEN, USA) using terminal deoxynucleotidyl

transferase (IBI, USA) to a specific activity of 7-10 x 10^8 cpm/μg and hybridised to the sections without pretreatment for 16-18 h at 42°C. The hybridisation mixture contained: 50% v/v formamide, 4 x SSC (1 x SSC = 0.15M NaCl and 0.015M sodium-citrate), 1 x Denhardt's solution (0.02% each of polyvinyl-pyrrolidone, BSA and Ficoll), 1% v/v sarcosyl (N-lauroylsarcosine; Sigma), 0.02M phosphate buffer (pH 7.0), 10% w/v dextran sulfate (Pharmacia, Sweden), 250μg/ml yeast tRNA (Sigma), 500μg/ml sheared and heat denatured salmon sperm DNA (Sigma) and 200mM dithiothreitol (DTT; LKB, Sweden). In control sections, the specificity of both probes was checked by adding a 20-fold excess of unlabeled probe to the hybridisation mixture. In addition, adjacent sections were hybridised with a probe unrelated to this study which gave a different expression pattern. Following hybridisation the sections were washed several times in 1 x SSC at 55°C, dehydrated in ethanol and dipped in NTB2 nuclear track emulsion (Kodak, USA). After 3-5 weeks the sections were development in D-19 developer (Kodak, USA) and cover-slipped. In some cases, sections were opposed to an autoradiographic film (Beta-max autoradiography film Amersham Ltd, UK) prior to emulsion-dipping.

The four different probes gave identical hybridisation patterns in all tissues examined. Mouse VRF expression was detecting already in the E8 embryo, in which positive signal was recorded over structures most likely corresponding to the neuronal tube. In sagittal sections of E14 mouse embryo the strongest hybridisation signal was present over heart and in the nervous system, especially cerebral cortex (Figure 14A). A low level of expression was present in all other tissues. At a later gestational age, E17, a high mVRF mRNA signal was confined to he heart and brown fat tissue in the back and around the neck (Figure 14B). Clearly positive hybridisation signals were present in the gray of the spinal cord and in the tongue (Figure 14B). Expression in the cerebral cortex was clearly reduced compared to day 14. The weak background expression seen in the E14 embryo in for example muscle, had decreased at this gestational age. A strong mVRF mRNA hybridisation signal was present solely over the heart and in the brown fat in the young adult mice (Figure 14C). The signal over the heart was evenly distributed ove the entire ventricular wall, including the papillary muscles (Figure 14D). In sections of heart tissue hybridised with an

excess of cold probe, no specific labeling over background signal was recorded (Figure 14E).

Apart from the heart, mVRF mRNA signal was present over certain tissues on the outside of the thoracic cage that morphologically resembled brown fat. This was verified with sudan black counterstaining, which showed a strong staining in the same areas (Figure 15A and 15B). In transverse sections of adult mouse spinal cord, the mVRF probes gave a neuronal staining pattern over the gray matter (Figure 15C). Counterstaining with toluidine (Figure 15D) showed that motoneurons in the ventral horn (Figure 15C and 15D), interneurons (Figure 15C) in the deep part of the dorsal horn and around the central canal where to a large extent positive for mVRF mRNA.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

TABLE 3

Splice junctions of the murine VRF gene

5′ UTR*.....	Exon 1	>223bp	CCCAGgtacgtgcgt	Intron I	495bp
ttccccacagGCCCC	Exon 2	43bp	GAAAGgtaataatag	Intron II	288bp
ctgcccacagTGGTG	Exon 3	197bp	TGCAGgtaccagggc	Intron III	196bp
ctgagcacagATCCT	Exon 4	74bp	TGCAGgtgccagccc	Intron IV	182bp
ctcttttcagACCTA	Exon 5	36bp	GACAGattcttggtg	Intron V	191bp
ctcctcctagGGTTG	Exon 6	101bp		(no intron)	
CCCACTCCAGCCCCA	Exon 7	135bp	TGTAGgtaaggagtc	Intron VI	-2200bp
cactccccagGTGCC	Exon 8	394bp	AGAGATGGAGACACT		

Uppercase and lowercase letters denote exonic and intronic sequences respectively.

* Indicates that the 5′ end of exon 1 has not yet been determined.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH and AMRAD CORPORATION LIMITED

(ii) TITLE OF INVENTION: A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME - II

(iii) NUMBER OF SEQUENCES: 14

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(2) INFORMATION FOR SEO ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 649 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 17...589

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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            15                  20                  25

CCC ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC     145
Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe
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ATG GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG     193
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GAC ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA     241
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60                  65                  70                  75

TCC TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC     289
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                80                  85                  90

CTG GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG     337
Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met
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CGG ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA     385
Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu
        110                 115                 120

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Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln
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CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT      529
Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg
                160                 165                 170

TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC      577
Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp
            175                 180                 185

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
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Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..624

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC     143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
            35                  40                  45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC     191
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GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT     239
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Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys
            115                 120                 125

AAA AAG GAC AGT GCT GTG AAG CCA GAC AGG GCT GCC ACT CCC CAC CAC      431
Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His
        130                 135                 140

CGT CCC CAG CCC CGT TCT GTT CCG GGC TGG GAC TCT GCC CCC GGA GCA      479
Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala
    145                 150                 155

CCC TCC CCA GCT GAC ATC ACC CAT CCC ACT CCA GCC CCA GGC CCC TCT      527
Pro Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser
160                 165                 170                 175

GCC CAC GCT GCA CCC AGC ACC ACC AGC GCC CTG ACC CCC GGA CCT GCC      575
Ala His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala
                180                 185                 190

GCT GCC GCT GCC GAC GCC GCA GCT TCC TCC GTT GCC AAG GGC GGG GCT T    624
Ala Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
            195                 200                 205

AGAGCTCAAC CCAGACACCT GCAGGTGCCG GAAGCTGCGA AGGTGACACA TGGCTTTTCA    684

GACTCAGCAG GGTGACTTGC CTCAGAGGCT ATATCCCAGT GGGGGAACAA AGGGGAGCCT    744

GGTAAAAAAC AGCCAAGCCC CCAAGACCTC AGCCCAGGCA GAAGCTGCTC TAGGACCTGG    804

GCCTCTCAGA GGGCTCTTCT GCCATCCCTT GTCTCCCTGA GGCCATCATC AAACAGGACA    864

GAGTTGGAAG AGGAGACTGG GAGGCAGCAA GAGGGGTCAC ATACCAGCTC AGGGGAGAAT    924

GGAGTACTGT CTCAGTTTCT AACCACTCTG TGCAAGTAAG CATCTTACAA CTGGCTCTTC    984

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 207 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1               5                  10                  15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
            20                  25                  30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
    50                  55                  60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                85                  90                  95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125

Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg
    130                 135                 140

Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro
145                 150                 155                 160

Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala
                165                 170                 175

His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala
            180                 185                 190

Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
        195                 200                 205
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 993 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..566

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG        47
   Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln
    1               5                  10                  15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC        95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
                20                  25                  30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC       143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
            35                  40                  45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC       191
Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr
        50                  55                  60

GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT       239
Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
    65                  70                  75

GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC       287
Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
80                  85                  90                  95

CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG       335
Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu
                100                 105                 110

GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA       383
Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys
            115                 120                 125

AAA AAG GAC AGT GCT GTG AAG CCA GAT AGC CCC AGG CCC CTC TGC CCA       431
Lys Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro
        130                 135                 140
```

```
CGC TGC ACC CAG CAC CAC CAG CGC CCT GAC CCC CGG ACC TGC CGC TGC      479
Arg Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys
    145                 150                 155

CGC TGC CGA CGC CGC AGC TTC CTC CGT TGC CAA GGG CGG GGC TTA GAG      527
Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu
160                 165                 170                 175

CTC AAC CCA GAC ACC TGC AGG TGC CGG AAG CTG CGA AGG TGACACATGG      576
Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
                180                 185

CTTTTCAGAC TCAGCAGGGT GACTTGCCTC AGAGGCTATA TCCCAGTGGG GGAACAAAGG   636

GGAGCCTGGT AAAAAACAGC CAAGCCCCCA AGACCTCAGC CCAGGCAGAA GCTGCTCTAG   696

GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC CATCATCAAA   756

CAGGACAGAG TTGGAAGAGG AGACTGGGAG GCAGCAAGAG GGGTCACATA CCAGCTCAGG   816

GGAGAATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT CTTACAACTG   876

GCTCTTCCTC CCCTCACTAA GAAGACCCAA ACCTCTGCAT AATGGGATTT GGGCTTTGGT   936

ACAAGAACTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAAA AAAAAAA      993
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 188 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1               5                  10                  15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
            20                  25                  30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
    50                  55                  60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80
```

```
Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                 85                  90                  95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125

Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg
    130                 135                 140

Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg
145                 150                 155                 160

Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu
                165                 170                 175

Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
            180                 185
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 858 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..431

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG        47
   Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln
    1               5                  10                  15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC      95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
                20                  25                  30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC     143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
            35                  40                  45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC     191
Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr
        50                  55                  60
```

```
GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT      239
Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
     65                  70                  75

GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC      287
Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
 80                  85                  90                  95

CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG      335
Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu
                100                 105                 110

GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA      383
Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys
            115                 120                 125

AAA AAG GAC AGT GCT GTG AAG CCA GAT AGG TGC CGG AAG CTG CGA AGG      431
Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Cys Arg Lys Leu Arg Arg
        130                 135                 140

TGACACATGG CTTTTCAGAC TCAGCAGGGT GACTTGCCTC AGAGGCTATA TCCCAGTGGG    491

GGAACAAAGG GGAGCCTGGT AAAAAACAGC CAAGCCCCCA AGACCTCAGC CCAGGCAGAA    551

GCTGCTCTAG GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC    611

CATCATCAAA CAGGACAGAG TTGGAAGAGG AGACTGGGAG GCAGCAAGAG GGGTCACATA    671

CCAGCTCAGG GGAGAATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT    731

CTTACAACTG GCTCTTCCTC CCCTCACTAA GAAGACCCAA ACCTCTGCAT AATGGGATTT    791

GGGCTTTGGT ACAAGAACTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAAA    851

AAAAAAA                                                              858
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1               5                  10                  15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
            20                  25                  30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
    50                  55                  60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                85                  90                  95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125

Lys Asp Ser Ala Val Lys Pro Asp Arg Cys Arg Lys Leu Arg Arg
    130                 135                 140
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 910 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..305

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG        47
   Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln
    1               5                  10                  15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC        95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
                20                  25                  30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC       143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
            35                  40                  45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC       191
Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr
        50                  55                  60

GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT       239
Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
    65                  70                  75

GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC       287
Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
80                  85                  90                  95

CAA GTC CGG ATG CAG ACC TAAAAAAAAG GACAGTGCTG TGAAGCCAGA              335
Gln Val Arg Met Gln Thr
                100

CAGGGCTGCC ACTCCCCACC ACCGTCCCCA GCCCCGTTCT GTTCCGGGCT GGGACTCTGC     395

CCCCGGAGCA CCCTCCCCAG CTGACATCAC CCATCCCACT CCAGCCCCAG GCCCCTCTGC     455

CCACGCTGCA CCCAGCACCA CCAGCGCCCT GACCCCCGGA CCTGCCGCTG CCGCTGCCGA     515

CGCCGCAGCT TCCTCCGTTG CCAAGGGCGG GGCTTAGAGC TCAACCCAGA CACCTGCAGG     575

TGCCGGAAGC TGCGAAGGTG ACACATGGCT TTTCAGACTC AGCAGGGTGA CTTGCCTCAG     635
```

AGGCTATATC CCAGTGGGGA ACAAAGAGGA GCCTGGTAAA AAACAGCCAA GCCCCCAAGA 695

CCTCAGCCCA GGCAGAAGCT GCTCTAGGAC CTGGGCCTCT CAGAGGGCTC TTCTGCCATC 755

CCTTGTCTCC CTGAGGCCAT CATCAAACAG GACAGAGTTG GAAGAGGAGA CTGGGAGGCA 815

GCAAGAGGGG TCACATACCA GCTCAGGGGA GAATGGAGTA CTGTCTCAGT TTCTAACCAC 875

TCTGTGCAAG TAAGCATCTT ACAACTGGCT CTTCC 910

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
20 25 30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
35 40 45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
50 55 60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65 70 75 80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
85 90 95

Val Arg Met Gln Thr
100

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACCACCACCT CCCTGGGCTG GCATGTGGCA CGTGCATAAA CG 42

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGTTGTTTGA CCACATTGCC CATGAGTTCC ATGCTCAGAG GC 42

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCCTGGGG CTGGAGTGGG ATGGATGATG TCAGCTGG 38

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCGGGCAGAG GATCCTGGGG CTGTCTGGCC TCACAGCACT 40

DATED this 22nd day of December, 1995

THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH and AMRAD CORPORATION LIMITED

By Its Patent Attorneys

DAVIES COLLISON CAVE

FIGURE 1

```
TCGGGCCTCC GAAACC ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC        49
                  Met Asn Phe Leu Leu Ser Trp Val His Trp Ser
                    1               5                  10

CTT GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA       97
Leu Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala
            15                  20                  25

CCC ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC      145
Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe
        30                  35                  40

ATG GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG      193
Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val
    45                  50                  55

GAC ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA      241
Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro
60                  65                  70                  75

TCC TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC      289
Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly
                80                  85                  90

CTG GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG      337
Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met
            95                  100                 105

CGG ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA      385
Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu
        110                 115                 120

CAG CAC AAC AAA TGT GAA TGC AGA CCA AAG AAA GAT AGA GCA AGA CAA      433
Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln
    125                 130                 135

GAA AAT CCC TGT GGG CCT TGC TCA GAG CGG AGA AAG CAT TTG TTT GTA      481
Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val
140                 145                 150                 155

CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT      529
Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg
                160                 165                 170

TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC      577
Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp
            175                 180                 185

AAG CCG AGG CGG TGAGCCGGGC AGGAGGAAGG AGCCTCCCTC AGCGTTTCGG          629
Lys Pro Arg Arg
        190

GAACCAGATC TCTCACCAGG                                                649
```

FIGURE 2

```
CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG          47
   Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln
     1               5                  10                  15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC          95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
                20                  25                  30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC         143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
            35                  40                  45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC         191
Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr
        50                  55                  60

GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT         239
Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
    65                  70                  75

GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC         287
Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
80                  85                  90                  95

CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG         335
Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu
                100                 105                 110

GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA         383
Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys
            115                 120                 125

AAA AAG GAC AGT GCT GTG AAG CCA GAC AGG GCT GCC ACT CCC CAC CAC         431
Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His
        130                 135                 140

CGT CCC CAG CCC CGT TCT GTT CCG GGC TGG GAC TCT GCC CCC GGA GCA         479
Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala
    145                 150                 155

CCC TCC CCA GCT GAC ATC ACC CAT CCC ACT CCA GCC CCA GGC CCC TCT         527
Pro Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser
160                 165                 170                 175

GCC CAC GCT GCA CCC AGC ACC ACC AGC GCC CTG ACC CCC GGA CCT GCC         575
Ala His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala
                180                 185                 190

GCT GCC GCT GCC GAC GCC GCA GCT TCC TCC GTT GCC AAG GGC GGG GCT T       624
Ala Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
            195                 200                 205
```

FIGURE 2 (continued...)

```
AGAGCTCAAC CCAGACACCT GCAGGTGCCG GAAGCTGCGA AGGTCACACA TGGCTTTTCA    684

GACTCAGCAG GGTGACTTGC CTCAGAGGCT ATATCCCAGT GGGGGAACAA AGGGGAGCCT    744

GGTAAAAAAC AGCCAAGCCC CCAAGACCTC AGCCCAGGCA GAAGCTGCTC TAGGACCTGG    804

GCCTCTCAGA GGGCTCTTCT GCCATCCCTT GTCTCCCTGA GGCCATCATC AAACAGGACA    864

GAGTTGGAAG AGGAGACTGG GAGGCAGCAA GAGGGGTCAC ATACCAGCTC AGGGGAGAAT    924

GGAGTACTGT CTCAGTTTCT AACCACTCTG TGCAAGTAAG CATCTTACAA CTGGCTCTTC    984

CTCCCCTCAC TAAGAAGACC CAAACCTCTG CATAATGGGA TTTGGGCTTT GGTACAAGAA   1044

CTGTGACCCC CAACCCTGAT AAAAGAGATG GAAGGAAAAA AAAAAAAAAA              1094
```

FIGURE 3

```
>VEGF_HUMAN VEGF_HUMAN  VASCULAR ENDOTHELIAL GROWTH FACTOR PRECURSOR (VEGF)
            (VASCULAR 215 AA.
            Length = 215

 Score = 181 (92.4 bits), Expect = 6.4e-20, P = 6.4e-20
 Identities = 33/75 (44%), Positives = 48/75 (64%)

Query:    31 HQRKVVSWIDVYTRATCQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECV 90
             +++ VV  +DVY R+ C+P E +V +  E    +      PSCV + RCGGCC D+GLECV
Sbjct:    36 NHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECV 95

Query:    91 PTGQHQVRMQILMIR 105
             PT +  + MQI+ I+
Sbjct:    96 PTEESNITMQIMRIK 110

 Score = 76 (38.8 bits), Expect = 0.0011, Poisson P(2) = 9.1e-12
 Identities = 12/19 (63%), Positives = 16/19 (84%)

Query:   110 QLGEMSLEEHSQCECRPKK 128
             ++GEMS  +H+ CECRPKK
Sbjct:   116 HIGEMSFLQHNKCECRPKK 134

 Score = 72 (36.8 bits), Expect = 0.0046, Poisson P(3) = 3.6e-18
 Identities = 14/21 (66%), Positives = 15/21 (71%)

Query:   202 RCQGRGLELNPDTCRCRKLRR 222
             RC +R LELN  TCRC K RR
Sbjct:   195 RCKARQLELNERTCRCDKPRR 215

 Score = 46 (23.5 bits), Expect = 47., Poisson P(4) = 7.3e-10
 Identities = 6/10 (60%), Positives = 9/10 (90%)

Query:   187 DPRTCRCRCR 196
             DP+TC+C C+
Sbjct:   181 DPQTCKCSCK 190
```

FIGURE 4

Gap Weight: 3.000 Average Match: 1.000
Length Weight: 0.100 Average Mismatch: -0.900
Quality: 100.9 Length: 739
Ratio: 0.175 Gaps: 30
Percent Similarity: 69.703 Percent Identity: 69.703

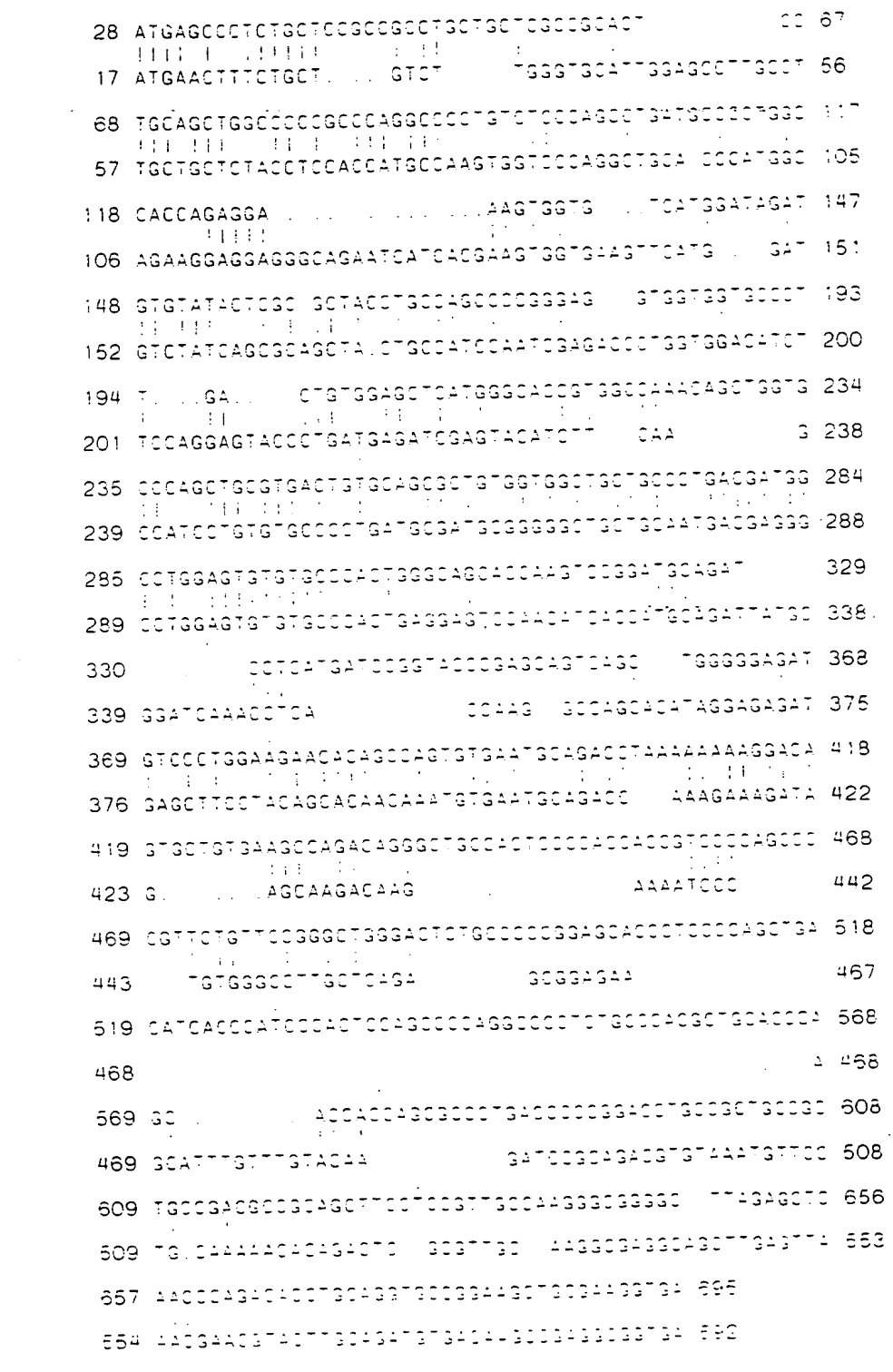


FIGURE 5

165SOMSQ.MSF.msf MSF: 687 Type: D Tuesday, June 20, 1995 Check: 3140

```
                1                                                                                                   80
    VEGF165     ATGAACTTTCTGCTGTCTTGGGTGCATTGGAGCCTTGCCTTGCTGCTCTACCTCCACCATGCCAAGTGGTCCCAGGCTG.
      SOM175    ATGAGCCCTCTGCTCCGCCGCCTGCTGCTCGCCGCACTCCTGCAGCTGGCCCCCGCCCAGGCCCCTGTCTCCCAGCCTGA
    SOM175-e6   ATGAGCCCTCTGCTCCGCCGCCTGCTGCTCGCCGCACTCCTGCAGCTGGCCCCCGCCCAGGCCCCTGTCTCCCAGCCTGA
  SOM175-e6&7   ATGAGCCCTCTGCTCCGCCGCCTGCTGCTCGCCGCACTCCTGCAGCTGGCCCCCGCCCAGGCCCCTGTCTCCCAGCCTGA
    SOM175-e4   ATGAGCCCTCTGCTCCGCCGCCTGCTGCTCGCCGCACTCCTGCAGCTGGCCCCCGCCCAGGCCCCTGTCTCCCAGCCTGA

                81                                                                                                  160
    VEGF165     CACCCATGGCAGAAGGAGGAGGGCAGAATCATCACGAAGTGGTGAAGTTCATGGATGTCTATCAGCGCAGCTACTGCCAT
      SOM175    TGCCCCTGGCCACCAGAGGAAAGTGGTGTCATGGATAGATGTGTATACTCGCG.....CTACCTGC.CAGCC.CCGGGAG
    SOM175-e6   TGCCCCTGGCCACCAGAGGAAAGTGGTGTCATGGATAGATGTGTATACTCGCG.....CTACCTGC.CAGCC.CCGGGAG
  SOM175-e6&7   TGCCCCTGGCCACCAGAGGAAAGTGGTGTCATGGATAGATGTGTATACTCGCG.....CTACCTGC.CAGCC.CCGGGAG
    SOM175-e4   TGCCCCTGGCCACCAGAGGAAAGTGGTGTCATGGATAGATGTGTATACTCGCG.....CTACCTGC.CAGCC.CCGGGAG

                161                                                                                                 240
    VEGF165     CCAATCGAGACCCTGGTGGACATCTTCCAGGAGTACCCTGATGAGATCGAGTACATCTTCAAGCCATCCTGTGTGCCCCT
      SOM175    GTGGTGGTGCCCTTGACTG.TGGAGCTCATGGGCACCGTGGCCAAAC..AGCTGGTGCCCAG......CTGCGTGACTGT
    SOM175-e6   GTGGTGGTGCCCTTGACTG.TGGAGCTCATGGGCACCGTGGCCAAAC..AGCTGGTGCCCAG......CTGCGTGACTGT
  SOM175-e6&7   GTGGTGGTGCCCTTGACTG.TGGAGCTCATGGGCACCGTGGCCAAAC..AGCTGGTGCCCAG......CTGCGTGACTGT
    SOM175-e4   GTGGTGGTGCCCTTGACTG.TGGAGCTCATGGGCACCGTGGCCAAAC..AGCTGGTGCCCAG......CTGCGTGACTGT

                241                                                                                                 320
    VEGF165     GATGCGATGCGGGGGCTGCTGCAATGACGAGGGCCTGGAGTGTGTGCCCACTGAGGAGTCCAACATCACCATGCAGATTA
      SOM175    GCAGCGCTGTGGTGGCTGCTGCCCTGACGATGGCCTGGAGTGTGTGCCCACTGGGCAGCACCAAGTCCGGATGCAGATCC
    SOM175-e6   GCAGCGCTGTGGTGGCTGCTGCCCTGACGATGGCCTGGAGTGTGTGCCCACTGGGCAGCACCAAGTCCGGATGCAGATCC
  SOM175-e6&7   GCAGCGCTGTGGTGGCTGCTGCCCTGACGATGGCCTGGAGTGTGTGCCCACTGGGCAGCACCAAGTCCGGATGCAGATCC
    SOM175-e4   GCAGCGCTGTGGTGGCTGCTGCCCTGACGATGGCCTGGAGTGTGTGCCCACTGGGCAGCACCAAGTCCGGATGCAGA...

                321                                                                                                 400
    VEGF165     TGCGGATCAAACCTCACCAAGGCCAGCACATAGGAGAGATGAGCTTCCTACAGCACAACAAATGTGAATGCAGACC...A
      SOM175    TCATGATCCGG...TACCCGAGCAGTCAGCTGGGGGAGATGTCCCTGGAAGAACACAGCCAGTGTGAATGCAGACCTAAA
    SOM175-e6   TCATGATCCGG...TACCCGAGCAGTCAGCTGGGGGAGATGTCCCTGGAAGAACACAGCCAGTGTGAATGCAGACCTAAA
  SOM175-e6&7   TCATGATCCGG...TACCCGAGCAGTCAGCTGGGGGAGATGTCCCTGGAAGAACACAGCCAGTGTGAATGCAGACCTAAA
    SOM175-e4   ..........................................................................CCTAAA

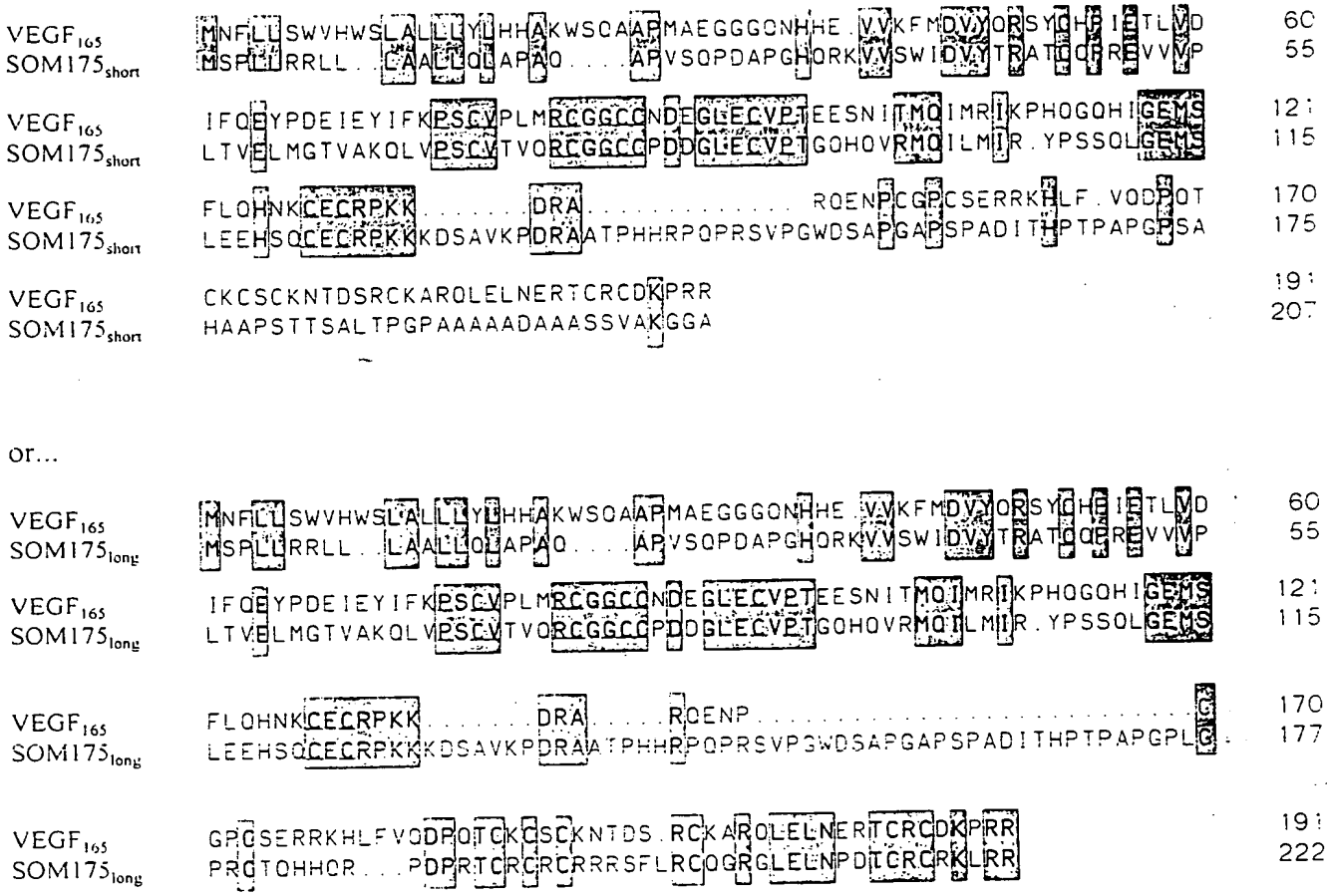
                401                                                                                                 480
    VEGF165     AAGAAAGATAG........AGCAAGACAAGAA....AATCCCTGTGG.............GCCTTGCTCAGAGCGGAGA
      SOM175    AAAAAGGACAGTGCTGTGAAGCCAGACAGGGCTGCCACTCCCCACCACCGTCCCCAGCCCCGTTCTGTTCCGGGCTGGGA
    SOM175-e6   AAAAAGGACAGTGCTGTGAAGCCAGATAG.....................................................
  SOM175-e6&7   AAAAAGGACAGTGCTGTGAAGCCAGATAG.....................................................
    SOM175-e4   AAAAAGGACAGTGCTGTGAAGCCAGACAGGGCTGCCACTCCCCACCACCGTCCCCAGCCCCGTTCTGTTCCGGGCTGGGA

                481                                                                                                 560
    VEGF165     ..........AAGCA.....................................................TTTGTT.....TGTAC..A
      SOM175    CTCTGCCCCCGGAGCACCCTCCCCAGCTGACATCACCCATCCCACTCCAGCCCCAGGCCCCTCTGCCCACGCTGCACCCA
    SOM175-e6   ..................................................CCCCAGGCCCCTCTGCCCACGCTGCACCCA
  SOM175-e6&7   ..........................................................................
    SOM175-e4   CTCTGCCCCCGGAGCACCCTCCCCAGCTGACATCACCCATCCCACTCCAGCCCCAGGCCCCTCTGCCCACGCTGCACCCA

                561                                                                                                 640
    VEGF165     A..............GATCCGCAGACGTGTAAATGTTCCTGCAAAAAC.ACAGACTCG..CGTTGCAAGGCGAGGCAGC
      SOM175    GCACCACCAGCGCCCTGACCCCCGGACCTGCCGCTGCCGCTGCCGACGCCGCAGCTTCCTCCGTTGCCAAGGGCGGGGCT
    SOM175-e6   GCACCACCAGCGCCCTGACCCCCGGACCTGCCGCTGCCGCTGCCGACGCCGCAGCTTCCTCCGTTGCCAAGGGCGGGGCT
  SOM175-e6&7   ..........................................................................
    SOM175-e4   GCACCACCAGCGCCCTGACCCCCGGACCTGCCGCTGCCGCTGCCGACGCCGCAGCTTCCTCCGTTGCCAAGGGCGGGGCT

                641                                                                 687
    VEGF165     TTGAGTTAAACGAACGTACTTGCAGATGTGACAAGCCGAGGCGGTGA
      SOM175    TAGAGCTCAACCCAGACACCTGCAGGTGCCGGAAGCTGCGAAGGTGA
    SOM175-e6   TAGAGCTCAACCCAGACACCTGCAGGTGCCGGAAGCTGCGAAGGTGA
  SOM175-e6&7   ......................GTGCCGGAAGCTGCGAAGGTGA
    SOM175-e4   TAGAGCTCAACCCAGACACCTGCAGGTGCCGGAAGCTGCGAAGGTGA
```

FIGURE 6



Areas of 100% homology are boxed and conserved residues thought to be involved in homodimerisation are underlined. The VEGF sequence depicted includes the 26 amino acid leader sequence (removal of which gives rise to mature $VEGF_{165}$) giving a total length of 191 amino acids.

Homology of SOM175 to $VEGF_{165}$ is 27% (33%) at the protein level, however within this are blocks of 100% homology. In particular, many structural residues are conserved including those thought to be involved in homodimerisation of VEGF (by comparison with PDGF).

ie. Cysteine-47
Proline-70, Cysteine-72, Valine-74
Arginine-77, Cysteine-78, Glycine-80, Cysteines-81 & 82
Cysteine-89, Proline-91
Cysteines 122 & 124

Splice variants of SOM175

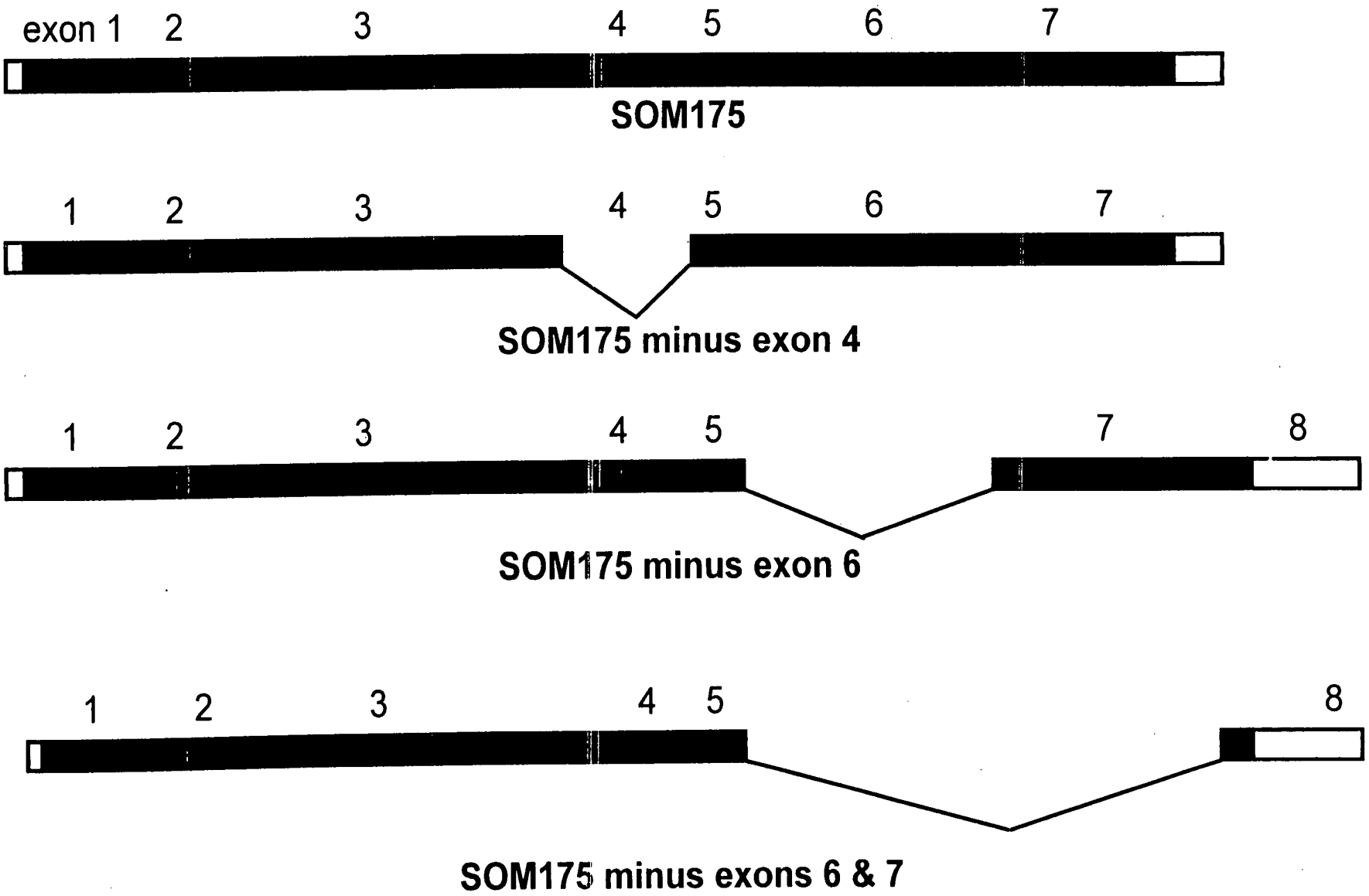


FIGURE 7

FIGURE 8A

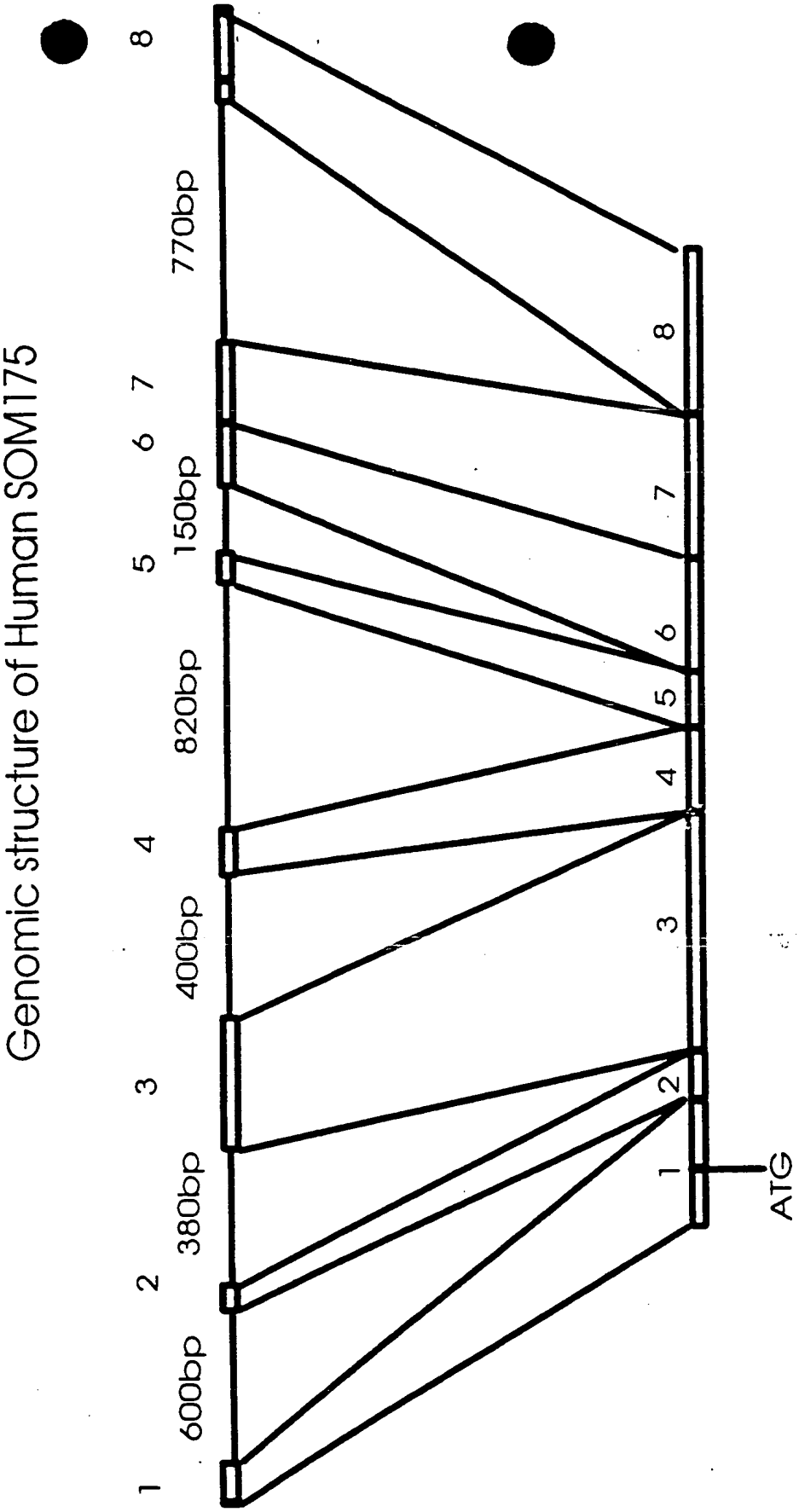

Genomic structure of Human SOM175
1
2
3
4
5
6
7
8
600bp
380bp
400bp
820bp
150bp
770bp
ATG

FIGURE 8B

5'UTR... **ATG**AGG	*Exon 1 (60bp)	GGCCAG gtacg gagg
tctcccacag GCCCCT	Exon 2 (43bp)	GGAAAG aatacttaca
tctgctccca TGGTGT	Exon 3 187bp)	ATGCAG gtccgagctg
ctgaatacag ATCCTC	Exon 4 (73bp)	ATGCAG gtgtcaggca
acttttcaag ACCTAA	Exon 5 (34bp)	AGACAG gtgagtcttt
ctcctccgta GGCTGC	Exon 6 (101bp)	CTCCAG ccccaggccc
cccactccag CCCCAG	Exon 7 (109bp)	ACCCAG acacctgtag
ccctgctcag GTGCCG	*Exon 8 (22bp)	AGG**TGA** ...3'UTR

FIGURE 9

```
-163  gcacgagctcaggccgtcgctgcggcgctgcgttgcgctgcctgcgcccagggctcggga
-103  gggggccgcggaggagccgcccctgcgccccgccccgggtccccgggtccgcgccatgg
 -43  ggcggctctggctgacccccccccacaccgccgggctagggcccgATGAGCCCCCTGCTG
                                                   M  S  P  L  L    -17

  16  CGTCGCCTGCTGCTTGTTGCACTGCTGCAGCTGGCTCGCACCCAGGCCCCTGTGTCCCAG
       R  R  L  L  L  V  A  L  L  Q  L  A  R  T  Q  A  P  V  S  Q     4

  76  TTTGATGGCCCCAGTCACCAGAAGAAAGTGGTGCCATGGATAGACGTTTATGCACGTGCC
       F  D  G  P  S  H  Q  K  K  V  V  P  W  I  D  V  Y  A  R  A    24

 136  ACATGCCAGCCCAGGGAGGTGGTGGTGCCTCTGAGCATGGAACTCATGGGCAATGTGGTC
       T  C  Q  P  R  E  V  V  V  P  L  S  M  E  L  M  G  N  V  V    44

 196  AAACAACTAGTGCCCAGCTGTGTGACTGTGCAGCGCTGTGGTGGCTGCTGCCCTGACGAT
       K  Q  L  V  P  S  C  V  T  V  Q  R  C  G  G  C  C  P  D  D    64

 256  GGCCTGGAATGTGTGCCCACTGGGCAACACCAAGTCCGAATGCAGATCCTCATGATCCAG
       G  L  E  C  V  P  T  G  Q  H  Q  V  R  M  Q  I  L  M  I  Q    84

 316  TACCCGAGCAGTCAGCTGGGGGAGATGTCCCTGGGAGAACACAGCCAATGTGAATGCAGA
       Y  P  S  S  Q  L  G  E  M  S  L  G  E  H  S  Q  C  E  C  R   104

 376  CCTAAAAAAAAGGAGAGTGCTGTGAGGCCAGACAGGGTTGCCATACCCCACCACCGTCCC
       P  K  K  K  E  S  A  V  R  P  D  R  V  A  I  P  H  H  R  P   124

 436  CAGCCCCGCTCTGTTCCGGGCTGGGACTCTACCCCGGGAGCACCCTCCCCAGCTGACATC
       Q  P  R  S  V  P  G  W  D  S  T  P  G  A  P  S  P  A  D  I   144

 496  ATCCATCCCACTCCAGCCCCAGGATCCTCTGCCCGCCTTGCACCCAGCGCCGCCAACGCC
       I  H  P  T  P  A  P  G  S  S  A  R  L  A  P  S  A  A  N  A   164
                   S  P  R  I  L  C  P  P  C  T  Q  R  R  Q  R      130

 556  CTGACCCCCGGACCTGCCGTTGCCGCTGTAGACGCCGCCGCTTCCTCCATTGCCAAGGGC
       L  T  P  G  P  A  V  A  A  V  D  A  A  A  S  S  I  A  K  G   184
       P  D  P  R  T  C  R  C  R  C  R  R  R  R  F  L  H  C  Q  G   150

 616  GGGGCTTAGAGCTCAACCCAGACACCTGTAGGTGCCGGAAGCCGCGAAAGTGAcaagctg
       G  A  *                                                       186
       R  G  L  E  L  N  P  D  T  C  R  C  R  K  P  R  K  *         167

 676  ctttccagactccacgggcccggctgcttttatggccctgcttcacagggagaagagtgg
 736  agcacaggcgtaacctcctcagtctgggaggtcactgccccaggacctggacctttaga
 796  gagctctctcgccatcttttatctcccagagctgccatctaacaattgtcaaggaacctc
 856  atgtctcacctcaggggccagggtactctctcacttaaccaccctggtcaagtgagcatc
 916  ttctggctggctgtctcccctcactatgaaaaccccaaacttctaccaataacgggattt
 976  gggttctgttatgataactgtgacacacacacacactcacactct gataaa agagatgga
1036  gacactaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
```

FIGURE 10

A

```
hVRF167   -21 MSPLLRRLLLAALLQLAPAQAPVSQPDAPGHQRKVVSWIDVYTRATCQPR 29
mVRF167   -21 MSPLLRRLLLVALLQLARTQAPVSQFDGPSHQKKVVPWIDVYARATCQPR 29

hVRF167    30 EVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQ 79
mVRF167    30 EVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQ 79

hVRF167    80 ILMIRYPSSQLGEMSLEEHSQCECRPKKKDSAVKPDSPRPLCPRCTQHHQ 129
mVRF167    80 ILMIQYPSSQLGEMSLGEHSQCECRPKKKESAVRPDSPRILCPPCTQRRQ 129

hVRF167   130 RPDPRTCRCRCRRRSFLRCQGRLELNPDTCRCRKLRR* 167
mVRF167   130 RPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK* 167
```

B

```
hVRF186   116 RAATPHHRPQPRSVPGWDSAPGAPSPADITHPTPAPGPSAHAAPSTTSAL  165
mVRF186   116 RVAIPHHRPQPRSVPGWDSTPGAPSPADIIHPTPAPGSSARLAPSAANAL  165

hVRF186   166 TPGPAAAAADAAASSVAKGGA* 186
mVRF186   166 TPGPAVAAVDAAASSIAKGGA* 186
```

FIGURE 11

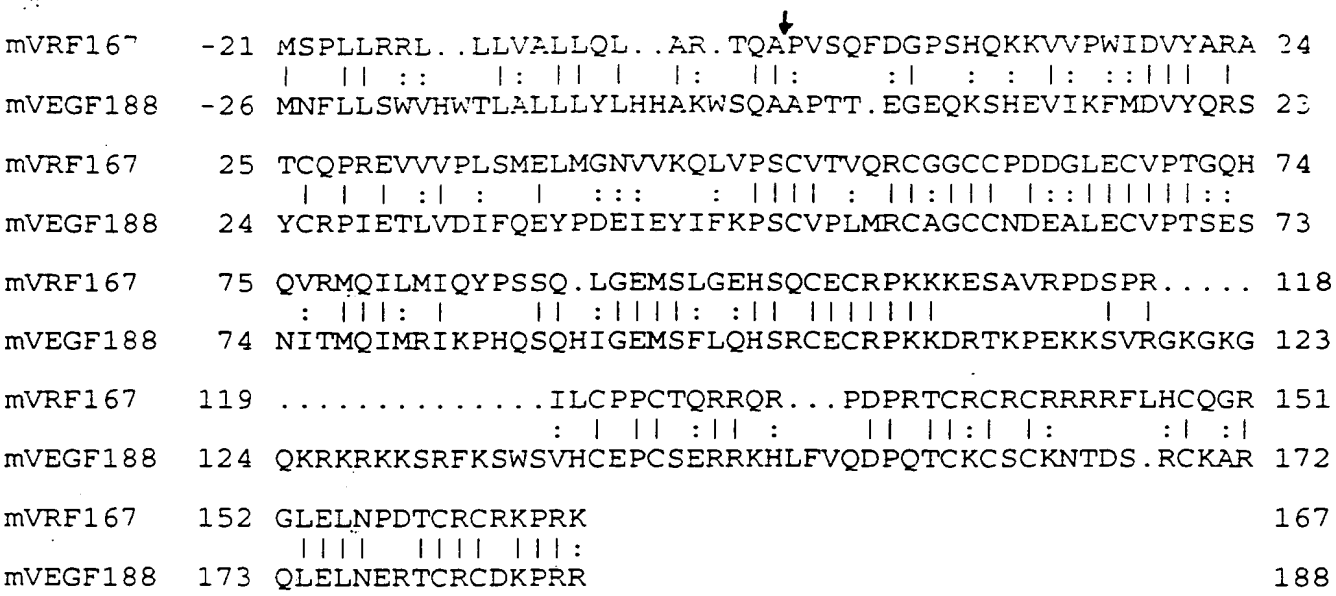

mVRF167 -21 MSPLLRRL..LLVALLQL..AR.TQAPVSQFDGPSHQKKVVPWIDVYARA 24
mVEGF188 -26 MNFLLSWVHWTLALLLYLHHAKWSQAAPTT.EGEQKSHEVIKFMDVYQRS 23
mVRF167 25 TCQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQH 74
mVEGF188 24 YCRPIETLVDIFQEYPDEIEYIFKPSCVPLMRCAGCCNDEALECVPTSES 73
mVRF167 75 QVRMQILMIQYPSSQ.LGEMSLGEHSQCECRPKKKESAVRPDSPR..... 118
mVEGF188 74 NITMQIMRIKPHQSQHIGEMSFLQHSRCECRPKKDRTKPEKKSVRGKGKG 123
mVRF167 119 .....ILCPPCTQRRQR...PDPRTCRCRCRRRRFLHCQGR 151
mVEGF188 124 QKRKRKKSRFKSWSVHCEPCSERRKHLFVQDPQTCKCSCKNTDS.RCKAR 172
mVRF167 152 GLELNPDTCRCRKPRK 167
mVEGF188 173 QLELNERTCRCDKPRR 188

FIGURE 12

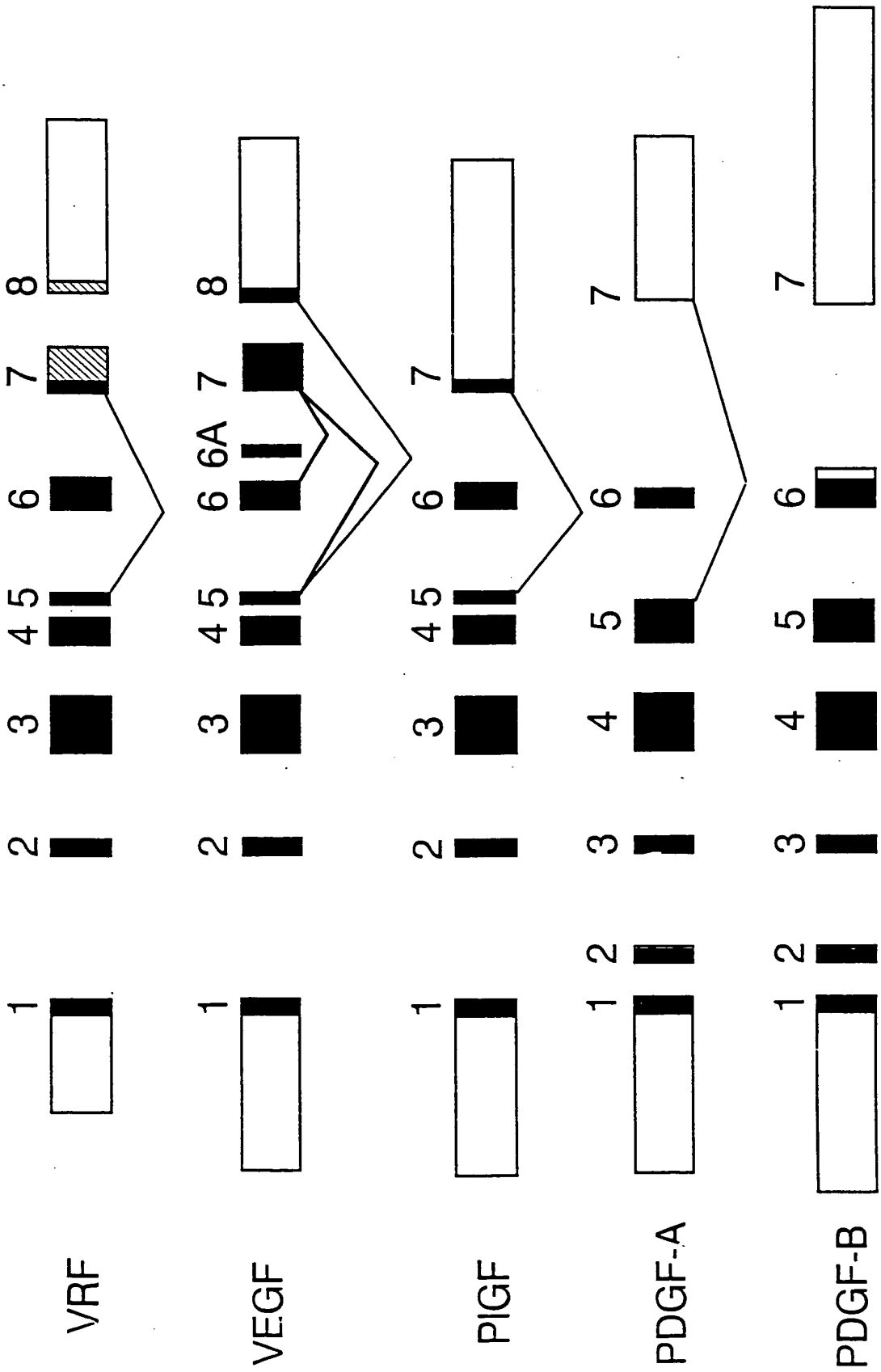
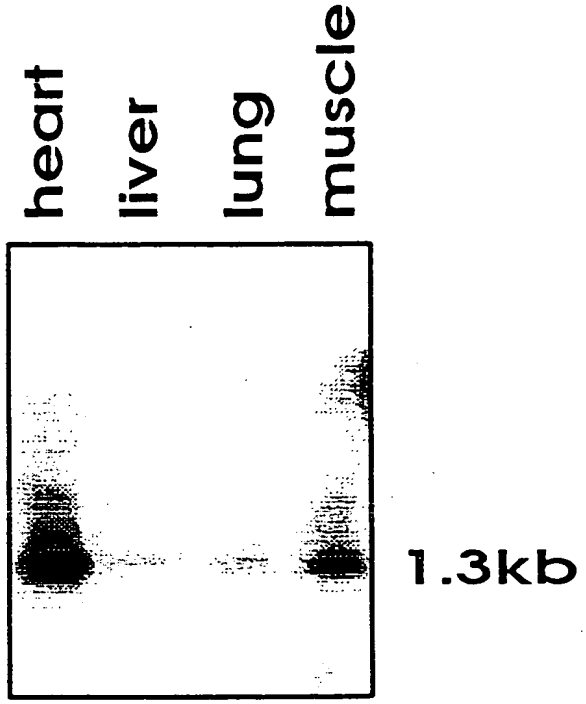


FIGURE 13



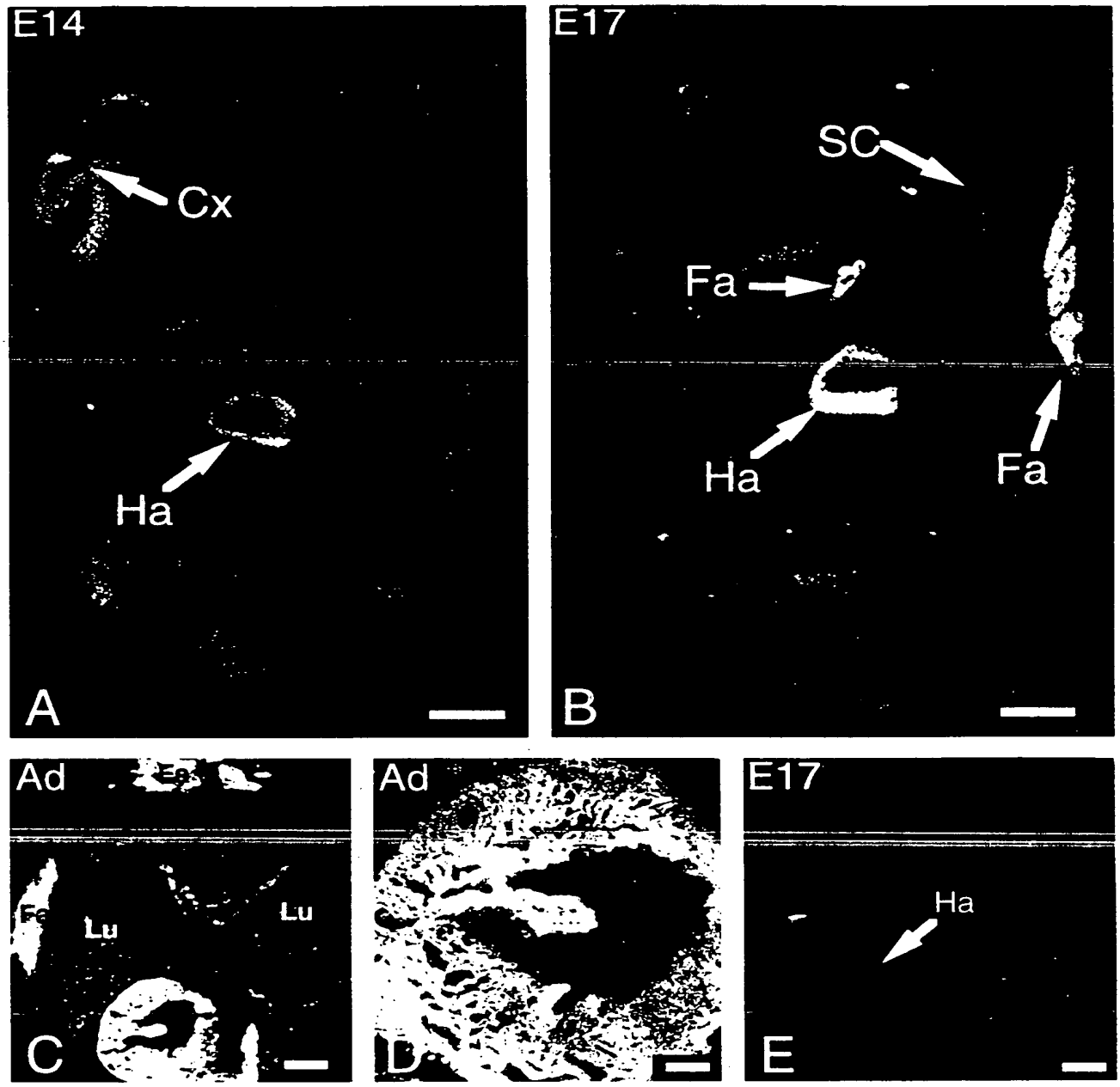


FIGURE 13